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Review article

How can the commercial potential of microalgae from the *Dunaliella* genus be improved? The importance of nucleotide metabolism with a focus on nucleoside diphosphate kinase (NDPK)

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ABSTRACT

Over recent decades, wide-ranging research has been focused on algae due to their potential applications, e.g., for biomass, extract and "third"-generation biofuel production. In particular, microalgae, which are very fast-growing organisms with low nutrient requirements, are ideal research objects. This review focuses on one of the most significant microalgae genus, i.e., *Dunaliella*, which includes *D. salina* and *D. tertiolecta*. The valuable components of these unicellular eukaryotes include glycerol, carotenoids (such as zeaxanthin and β-carotene) and many others, which mainly accumulate when these microalgae are affected by stress conditions (i.e., hypersaline and oxidative stress). The rapid growth of these microalgae in artificial/natural systems certainly has to be based not only on nutrient uptake and photosynthesis rates but also on efficient nucleotide metabolism. Nucleoside diphosphate kinases (NDPKs), which are considered conserved housekeeping proteins responsible for nucleotide turnover, could play an important role in this context, together with proper mitochondrial and chloroplast function. A more detailed understanding of the biochemical nature of *Dunaliella* spp. will help optimize the massive cultivation of these microorganisms. Ultimately, it will facilitate the greater use of coastal waters to multiply *Dunaliella* biomass production significantly. This strategy will save freshwater resources, e.g., in obtaining land plant oils to make biofuels. Greater access to different algal bioactive compounds may support the treatment of various diseases, such as asthma, eye diseases, cancer, and viral infections.

1. Introduction

Microalgae are mostly autotrophic unicellular eukaryotes that are typically found in freshwater, brackish and marine ecosystems [1,2]. For years, >40,000 species of these organisms have been considered for use as alternative sources of food, biofuels and medicines [3,4]. Currently, hundreds of microalgae species are cultivated both under closed and open aquacultures, but significant biomass for industry is produced from fewer than 20 taxa [5,6]. It is estimated that the microalga-based global market will reach more than 56.5 billion American dollars by 2027, according to a compound annual growth rate of >6% from 2019 to 2027 [7]. Therefore, modern, developed societies should more carefully consider the benefits of microalga-derived goods. Recently, the greatest production performance of microalgae in artificial systems has been associated with three genus: *Chlorella*, *Dunaliella*, and *Tetradesmus* [3,4,8].

In this paper, we highlight the scientific, medicinal and industrial and, thus, commercial potential of a unique group of microalgae — *Dunaliella* spp. The rapid growth rate, effective biogen assimilation, fast adaptability to stresses (mostly abiotic), and variety of useful biocompounds observed in representatives of this genus present great potential for novel human applications [8,9]. Efficient energy transduction has to be a critical factor in the success of *Dunaliella* spp. evolution. Finally, nucleotide metabolism will be considered here, with an

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; crf-DS, carotenoid-rich fraction of *D. salina*; F_OF_1, two structurally and functionally distinct segments of ATP synthase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; Gy, gamma rays; NDPK, nucleoside diphosphate kinase; OXPHOS, oxidative phosphorylation; RNA, ribonucleic acid; ROS, reactive oxygen species; SAG, Sammlung von Algenkulturen der Universität Göttingen.

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emphasis on nucleoside diphosphate kinase (NDPK).

2. Taxonomy and description of *Dunaliella* spp.

Marine microalgae from the *Dunaliella* Teodoresco (1905) genus belong to the phylum *Chlorophyta* (green algae), order *Chlamydomonadales*, and family *Dunaliellaceae* [1,10]. Currently, the genus *Dunaliella* includes 26 distinct species, which are commonly accepted taxonomically. The best-recognized species of *Dunaliella* are *D. salina* (Dunal) Teodoresco 1905, *D. tertiolecta* Butcher 1959, and *D. viridis* Teodoresco 1905 [8].

Dunaliella spp. are planktonic, unicellular, photosynthetic, motile biflagellate algae [1,9–12]. The cells are radially symmetrical, slightly asymmetrical, ellipsoidal, oval, ovate or close to these shapes (Fig. 1). The two flagella (of equal length) may be even 2.5 times as long as the cell length. A single posterior chloroplast (often cup-shaped) occupies the majority of the cytosol and contains axial (nearly medial) or basal pyrenoids as well as stigma (in chloroplast lobe), which may not be clearly delineated. The anterior of the cell is colorless and free of chloroplast. Interestingly, all *Dunaliella* representatives lack a cell wall, which makes these organisms more flexible. The wallless form is an unusual feature that distinguishes *Dunaliella* spp. from other green microalgae. Such a unique property means that *Dunaliella* spp. can be readily digested by the majority of animals, including humans [8,13]. However, *Dunaliella* cells are not always completely naked as mucilaginous coat (containing glycoproteins) is sometimes evidenced [8,9,11,14]. This covering with a spongy structure, referred to as pericellular matrix, may be even approx. 20 times wider than the plasma membrane itself (based on the analysis of *D. salina*) [14]. Unfortunately, the composition of this layer is still poorly understood.

3. Structural, physiological and biochemical advantages of *Dunaliella* spp.

The cells of *Dunaliella* spp., which naturally lack wall layers, make them suitable model organisms for studying stress signaling [9,15,16]. The absence of a rigid cell wall (eliminating a significant physical barrier) makes these microalgae more penetrable to exogenous substances/molecules [8,17]. Therefore, osmoregulation, carbon metabolism, gene expression, photosynthesis rate and growth rate have been tested in the context of different environmental conditions, pollutants, and toxicants. Interestingly, under stress conditions, *Dunaliella* spp. enhance protein biosynthesis and accumulate low-molecular weight organic compounds (such as glycerol and carotenoids) as well as sugar-based biopolymers (e.g., starch) (Fig. 2) [14,18–22]. Thus, the potential of these microorganisms for use on an industrial scale has been noted. *D. salina* and *D. tertiolecta* are generally considered commercially important species [8,16,21], and numerous easy-to-grow *Dunaliella* species/strains are deposited in culture collections, e.g., Culture Collection of Algae (SAG) at Göttingen University, Germany [11].

4. Distribution and ecology of *Dunaliella* spp.

Species of the *Dunaliella* genus are generally found in all oceans and continental inland waters [1,9,11,23]. Most taxa are euryhaline (e.g., *D. tertiolecta*, *D. primolecta* Butcher 1959, *D. polymorpha* Butcher 1959, and *D. quartolecta* Butcher 1959) and thus occur in brackish to marine ecosystems. Therefore, these species are quite tolerant to salinity fluctuations. Moreover, some of them (e.g., *D. parva* W. Lerche 1937, *D. pseudosalina* Massjuk and Radchenko 1973, *D. salina* and *D. viridis*) inhabit hypersaline waters worldwide, including coastal lagoons and salt lakes. The global distribution of *Dunaliella* spp. is closely related to tolerance toward a wide range of environmental parameters (predominantly salinity, temperature, and light exposure); however, existence in

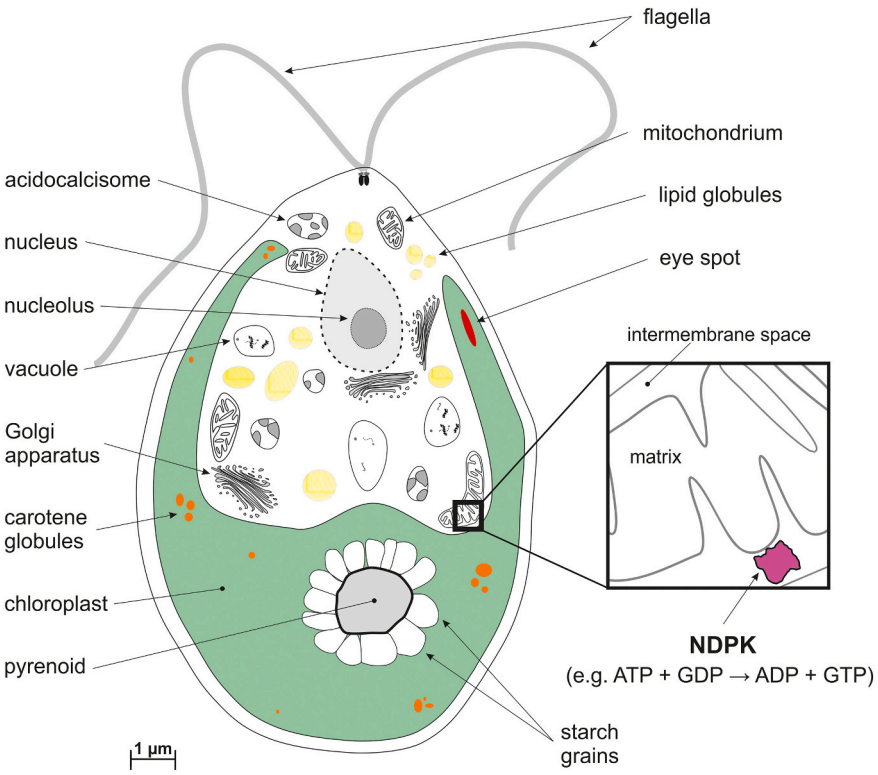

Fig. 1. Schematic ultrastructure of *Dunaliella* spp. β-Carotene is concentrated in small globules within the chloroplast, while lipid droplets are deposited in the cytosol. NDPK — nucleoside diphosphate kinase hexamer targeted to mitochondria (an example of a reaction catalyzed by this enzyme is shown — see also Section 6).

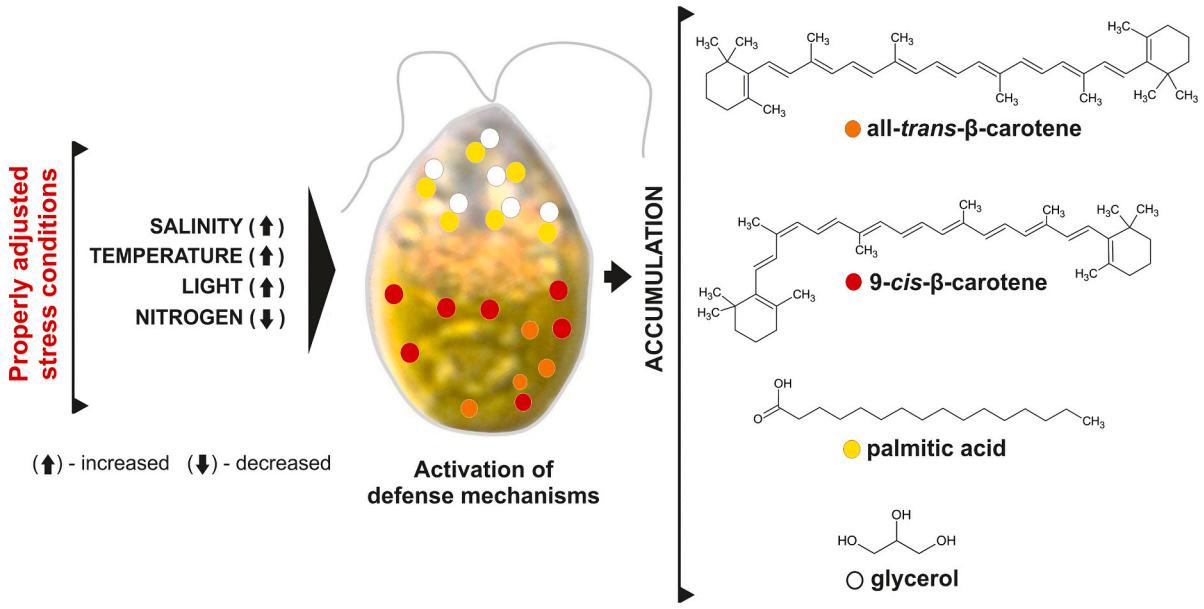

Fig. 2. Effect of stressful conditions on the increases in valuable organic compounds accumulated in *Dunaliella* spp. A microphotograph of *D. tertiolecta* (SAG 13.86) cell is shown as an example.

extreme habitats such as habitats with poor access to water is also possible. For example, some *Dunaliella* cells can exist for many years in naturally saturated salt basins, such as the Dead Sea (Israel/Jordan) and Great Salt Lake (USA), and even among damp crystals from coastal salt pans [23]. In addition, one acidophilic species (*D. acidophila* (Kalina) Massjuk 1971) was originally found in ecosystems with an extremely low pH (even <1.5) in Europe [24,25]. It must be kept in mind that in freshwater and marine phytoplankton communities, *Dunaliella* spp. are usually less well represented than other microalgal taxonomic groups, which is probably the reason for the poor understanding of *Dunaliella* ecology [9]. On the other hand, in hypersaline environments (>3.5%), *Dunaliella* spp. can form large-scale blooms known as "red tides", which occur mostly in the summertime. Under these circumstances, *Dunaliella* is the dominant microalgal genus in the ecosystem and is consequently a major producer of biomass.

5. Commercial potential of biomass and extracts derived from *Dunaliella* spp.

Only certain strains of *D. salina* and *D. tertiolecta* present biotechnological importance [8,15,26–28]. Specifically, the demand for alga-derived lipophilic compounds (such as carotenoids and triacylglycerides) is increasing each year [7]. The significant commercial potential of *Dunaliella* spp. is based on the stress-induced (mainly salinity, irradiance, and nitrogen depletion) intracellular accumulation of carotenoids (even >15%, dw, of which approx. 10% may constitute β-carotene) and triacylglycerides (even approx. 40%) in the form of easy-to-observe globules (Figs. 1 and 2) [9,14,26,29–31]. This kind of response is thought to protect negatively affected algae cells, for example, it could counteract excessive reactive oxygen species (ROS) generation. Increased amount of nonenzymatic (low-molecular weight)

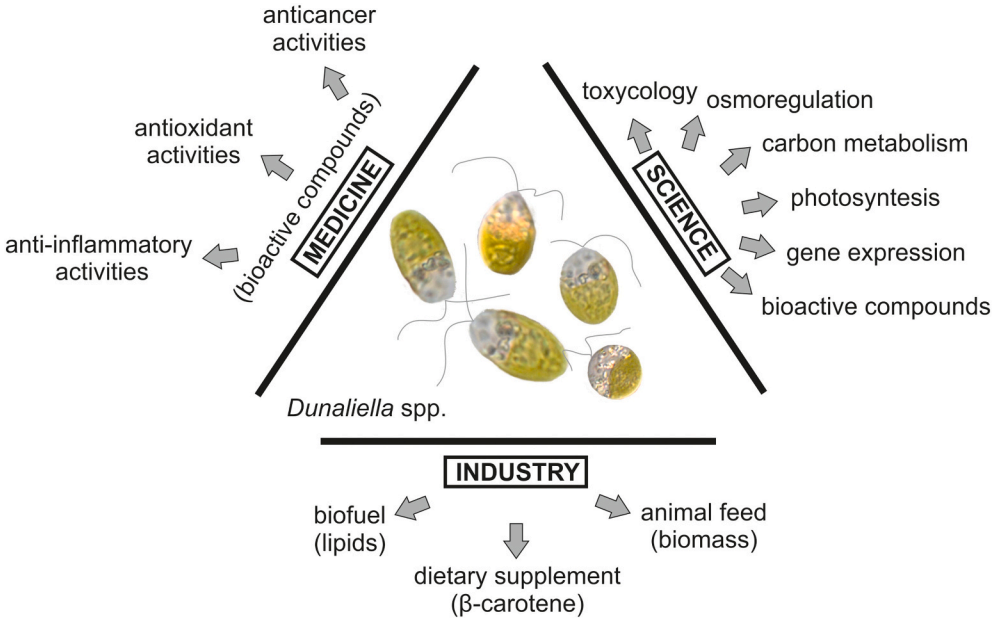

Fig. 3. Three main sectors using *Dunaliella* spp. biomass and extracts. Microphotographs of *D. tertiolecta* (SAG 13.86) cells are shown as an example.

antioxidants (such as carotenoids) together with increased activity/expression of some antioxidative enzymes (e.g., glutathione peroxidase) might be involved in the alleviation of oxidative stress [9,19,22]. Therefore, these nontoxic microalgae rich in bioactive compounds serve as a resource for the production of medicines and are promising material for biofuel technologies (Fig. 3) [27,32]. Functional ingredients from *Dunaliella* spp. have also been introduced in the gastronomy and cosmetic sectors [33–36].

Although *Dunaliella* spp. have been successfully grown in outdoor (open) and indoor (closed) cultivation systems [28,37], their viability and thus biomass production efficiency may be restricted by some abiotic stresses induced by anthropogenic pollutants accumulation in the environment [20,38–40]. Importantly, the concentration of heavy metals and common salts in *Dunaliella* biomass should always be determined before processing [41]. For this reason, harvesting *Dunaliella* cells from random places is not recommended. Only closed, controlled cultures are safe to operate and should thus be applied for the production of animal/human feed or as a source of bioactive compounds for pharmacology and cosmetology.

5.1. Effects of Dunaliella-derived preparations on animals

The influence of liquid and dry products extracted from *Dunaliella* cells has been tested on animals, including both invertebrate and vertebrate species, and in cell lines. *Dunaliella*-based preparations have received considerable attention because they may protect against some diseases or improve treatment. Currently, *D. salina*-based preparations are most commonly used for their beneficial effects because this microalga is an extremely rich natural source (the most abundant of all known) of β-carotene (approx. 10% in some strains) [9,29–31]. The amounts of all-*trans* and 9-*cis* isomers of β-carotene in *D. salina* are variable and depend on red light exposure which increases the pool of the 9-*cis* form [42]. It should be highlighted that 9-*cis*-β-carotene exhibits a stronger antioxidant capacity than all-*trans*-β-carotene, i.e., the *cis* bond is more reactive (has a greater affinity for free radicals) than the *trans* bond [43–45].

Interesting results were obtained by analyzing the effects of *D. salina* extracts on rat liver cells (line HSC-T6 and NRL clone 9) [46]. The natural mixture of various carotenoids, mainly consisting of all-*trans*-β-carotene and 9- or 9′-*cis*-β-carotene in proportion approx. 1:1 of *trans* to *cis* forms (~90% of the extract) resulted in a greater limitation of hepatotoxicity (artificially induced by cadmium chloride, $CdCl_2$) than all-*trans*-β-carotene alone. Similar ratio of all-*trans*-β-carotene and 9- or 9′-*cis*-β-carotene among total carotenoids (without extraction) of intact *D. salina* cells (orally administered as a water suspension of algae) were tested against murine leukemia (induced by intravenously injected cells of the WEHI-3 line) [47]. Three different doses of *D. salina* (184.5, 369, and 922.5 mg/kg body for 2–4 weeks) were shown to exert immunomodulatory effects *in vivo* (e.g., increased phagocytic activity by macrophages) and extended the life of leukemic mice. Interestingly, commercially available synthetic β-carotene (922.5 mg/kg body) may cause a similar effect to the lowest dose of carotenoid mixture from *D. salina* (184.5 mg/kg body). Another animal models confirmed that a diet enriched with *Dunaliella* preparations can positively affect the immune response and survival rate [48,49]. Specifically, food supplemented with a *Dunaliella* extract (with carotenoids such as all-*trans*-β-carotene, 9-*cis*-β-carotene, α-carotene and lutein in total concentration of ~10 μM) improved the mitochondrial function (cellular respiration) of common fruit fly males (*Drosophila melanogaster*) [48]. In particular, the total level of ATP increased by 26.5%, to which oxidative phosphorylation (OXPHOS)-dependent ATP synthesis is normally the major contributor. During the aging of these insects (both sexes), the most beneficial effect was also achieved with an ~10 μM mixed solution of different carotenoids (see above). Ultimately, an augmented whole-body metabolic rate (by 27% and measured *via* CO_2 production) observed upon this type of *Dunaliella* extract treatment favored the mobility (climbing ability) of flies and prolonged their lifespan (by up to 32.6% in the case of males). These experiments supported the view that carotenoids are associated with the amelioration of biological functions [50]. In the case of another invertebrate, the black tiger shrimp (*Penaeus monodon*), a proper diet containing *Dunaliella*-derived preparation, also had a beneficial effect on immune functions and survival [49]. The shrimp that were fed pellets (to satiation) with *D. salina* extract (containing 125, 200 or 300 mg β-carotene/kg diet) showed faster weight gain, increased resistance to white spot syndrome virus infection, and increased tolerance to habitat stress (dissolved oxygen deficiency). Curiously, the shrimp fed a diet supplemented with *D. salina*-derived ingredients showed a more reddish coloration after boiling (compared to the control) and thus must have accumulated carotenoids. Moreover, *in vivo* studies have shown that a diet enriched in *D. salina* biomass protects the central nervous system of rats [13]. The microalga meal contained an 8% β-carotene stereoisomer mixture (nearly 1:1 of 9-*cis*- and all-*trans*-forms). Specifically, the provision of a 1 g β-carotene/kg diet for one week counteracted hyperoxia-induced seizures correlated with ROS toxicity (i.e., the development of the disorder was delayed). Importantly, the protective effect is rather strongly limited for commercial (synthetic) all-*trans*-β-carotene or oxidized (aged) β-carotene from *D. salina* powder (0.3 ratio for 9-*cis*- to all-*trans*-forms), which were also administered together with a normal, well-balanced diet to rats. The active substance in this case is probably vitamin A (retinol), derived from its precursor, 9-*cis*-β-carotene. It has also been revealed that 9-*cis*-β-carotene is more directly linked with the antiperoxidative effect observed in rats *in vivo* than its all-*trans* counterpart [44]. In another study, doses of *D. salina* preparation (750 mg of endogenous substances within 3 days in total) were administered intragastrically to diabetic rats, but some of the obtained results were unexpected [51]. While a significant drop in triglycerides, and a slight drop in cholesterol in blood plasma were beneficial effects, increased hyperglycemia was a side effect. A similar approach demonstrated that a two-step treatment of rats with a *D. salina* biomass (daily 100 or 200 mg/kg body, 7 days in total) efficiently alleviated intestinal mucositis resulting from gamma-ray irradiation (6 Gy dose) [52]. Therefore, *D. salina* constituents (regardless of the dose tested) that protect the intestinal mucosal barrier through antioxidant and anti-inflammatory actions may aid in the recovery of oncological patients exposed to ionizing radiation. Moreover, the carotenoid-rich fraction of *D. salina* (crf-DS) administered orally (daily 150 mg/kg body for 6 weeks) showed therapeutic efficiency against some negative effects of obesity [53]. Specifically, the dietary supplementation of rats with obesity (induced by a high-fat diet) with crf-DS attenuated obesity-associated cardiac dysfunction (i.e., heart failure involving congestion and vascular fibrosis, which were symptoms observed when crf-DS was not administered). Interestingly, a controlled crf-DS diet and standard drug against obesity, i.e., orlistat (12 mg/kg body) as the lipase inhibitor, were practically equally effective in the protection of the heart. Similarly, forage support with oral tablets with an ~73% content of *D. salina* biomass (daily 100 mg/kg body for 4 weeks) can show an antifibrotic effect in rat liver [54]. An impact assessment of diet supplemented with *D. salina* preparation (containing β-carotene) against neoplastic changes (mammary tumors) was performed in virgin mice and showed the success of this approach [55,56]. In addition, an aqueous extract of *D. salina* intratumorally injected into mice with breast cancer showed a significant curative effect; i.e., tumor growth was restricted (probably *via* cytotoxic effects on cancer cells – based on *in vitro* studies with 4T1 mouse mammary tumor cells) [57]. Notably, clear therapeutic effects were observed with preparations acquired from *D. salina* cells grown under stress conditions, i.e., under high salinity (~11.7% NaCl), intense light (540 μmol photons/m^2/s^1) and a strongly limited nitrogen source.

The food safety aspects of dehydrated *D. salina* biomass (5 or 10% of forage) have been evaluated in multigenerational studies on rats [41]. Dietary supplementation with this biomass (2% β-carotene and 12% of other lipid compounds, 17% protein, 18% glycerol, 24% carbohydrate,

and 27% others) consistently administered for a year had a rather neutral influence on the growth, reproduction, behavior, appearance, and general pathology of animals from all examined generations (four in total). Intriguingly, both anti- and proinflammatory effects of *Dunaliella*-based preparations were noted at the highest dose (food with 10% microalgae biomass) within one year (based on first generation studies). While cases of chronic cholangitis and interstitial nephritis decreased, the number of individuals with focal bronchopneumonia significantly increased (explained as a possible side effect of *D. salina* powder irritating the respiratory system). There are generally no contraindications for the consumption of *Dunaliella* biomass by humans, which is supported by independent research on rats (and additionally on mice) [58]. The authors of this work found no organ failure (cardiac, renal, and hepatic toxicity) of high doses of *D. salina* powders (daily dose of up to 100 mg/kg of body weight for 3 months), which, as a side effect, significantly stimulated the synthesis of hemoglobin relative to the control groups. Therefore, *Dunaliella* spp. products may contribute to the treatment of various human diseases, such as anemia. In summary, the composition of tablets (containing proper solubilizers and bioenhancers) and the degree of fragmentation of the biomass (nondusting) will be conducive to the desired pro-health effect [41,54].

5.2. The potential use of Dunaliella-based preparations in the treatment of human diseases

The responses of various human cell lines to *Dunaliella*-derived preparations were also the subject of research. Different extracts of *D. tertiolecta* (after the lyophilization of microalgae followed by organic solvent treatment) were shown to exert potent antiproliferative activity against a human mammary cancer cell line (MCF-7) [59]. Although epoxycarotenoid (violaxanthin) is naturally produced and accumulates in *Dunaliella* cells only at low concentrations (approx. 0.06% w/w freeze-dried biomass), it has been shown to be a bioactive substance with strong anticancer effects (inducing apoptosis). Melanoma can also be cured by *D. salina*-derived preparations [60]. Ethanol extracts of algae cultivated under stress conditions (35% salinity and 4000 μmol photons/m^2/s^1 light intensity) were found to contain approx. 50% more β-carotene (compared to optimal conditions, i.e., 15% salinity and 100 μmol photons/m^2/s^1) and to be more effective against a human skin carcinoma cell line (A431) by increasing the cell death rate. These data are in accord with the findings of another report demonstrating the influence of *D. salina* ethanol extract on a human lung cancer cell line (A549) [61]. Cell cycle impairment and apoptosis were noted as antiproliferative effects of this type of preparation with approx. 6.9% (w/w) β-carotene. The remarkable biocatalyzator properties of the aqueous extract of *D. salina* (rich in phenols, flavonoids, and proteins) may be exploited for the formation and stabilization (as a reducing and capping agent) of gold and silver nanoparticles [62,63]. Such metal-biocompound aggregates are generally nontoxic to a normal human breast cell line (MCF-10A) but exert activity against a related cancer cell line (MCF-7). Targeting of active substances from *Dunaliella* extracts is probably improved thanks to Au/Ag nanoparticles. Therefore, *Dunaliella* spp. may represent a supportive tool in anticancer therapy applied as a nanomedicine.

The introduction of microalgal biomass for human health is continuously developing [64]. Tablets containing processed *Dunaliella* cells could possibly protect against/prevent the development of bronchoconstriction symptoms or even asthma syndromes [65]. For example, the intake of hard capsules containing 250 mg of nonmodified *D. salina* powder (with 8% β-carotene) three times a day for a week (approx. 60 mg/day of β-carotene, stereoisomer mixture) produced a promising result. Specifically, among 38 patients (aged 8 to 33) with a proven predisposition for exercise-induced bronchospasm, the negative post-exercise effect was partially abolished in 53% of the subjects (20 people) after such supplementation.

5.3. Optimization of Dunaliella biomass and extract composition for industrial purposes

Marine and freshwater biomass may be used in many branches of industry, but the exploitation of mass cultures of microalgae to obtain biofuels may be of particular importance for future applications (Fig. 3) [66–68]. It should be noted that some microalgae are much more effective oil producers than commonly known oil crops [69]. *Dunaliella* spp. might also be suitable for fuel oil manufacture [26,27,68–71]. Interestingly, the residual *Dunaliella* biomass after oil extraction could be further utilized for purposes such as bioethanol generation (using *Saccharomyces cerevisiae*) [70]. Biodiesel production from *Dunaliella* cells could also be indirectly supported by the integration of different factory bioreactors with the aim of carbon (derived from yeast alcohol fermentation) capture by microalgal cultures [71]. Such an approach, i.e., controlled bioassimilation of CO_2 (e.g., released during brewing) to increase the growth rate (and thus finally productivity) of algae, is in accord with global policy aimed at limiting CO_2 pollution.

As mentioned above, a protective effect against environmental stressors exerted on *Dunaliella* spp. is the overaccumulation of organic compounds (as a result of early rapid and long-term responses) such as carotenoids (e.g., α-carotene, β-carotene, lutein, and zeaxanthin), glycerol, fatty acids [e.g., α-linolenic (18:3), oleic (18:1), and palmitic (16:0) acids], and many others (Fig. 2) [18,71–74]. These robust defense strategies have ultimately been exploited for commercial purposes. As some *Dunaliella* spp. can easily adapt to diverse saline conditions; thus, they are ideal microorganisms for achieving controlled biosynthesis in both natural and artificial seawater. Interestingly, the presence of exogenous NaN_3 (sodium azide — toxic inorganic salt) at ~0.3–0.6% and increased salinity (~8.8–14.6% NaCl) may act synergistically to significantly increase the contents of some lipids in *D. tertiolecta* cells without severely compromising algae viability (after 12 days) [38]. However, NaN_3 may simultaneously cause clear decreases in chlorophyll and carotenoid pools. Similarly, toxic triethylamine (0.005–0.02% $C_6H_{15}N$) stimulates the formation of lipid globules in *D. tertiolecta* cells and negatively affects their chlorophyll content, carotenogenesis, and algae number (steep decreases) [39]. In this study, fatty acid profiling revealed the largest increase in palmitic acid (16:0) after treatment with this chemical inducer. Curiously, it has been reported that in *D. salina*, triethylamine (0.005%) at higher salinity (~8.8% NaCl) promotes maximal lycopene accumulation after 3 days of treatment [20]. Lycopene is a linear carotenoid and intermediate for α- and β-carotene formation. The decrease in cellular β-carotene concentration under such conditions could be explained by $C_6H_{15}N$-mediated inhibition of lycopene cyclase. Therefore, the positive feedback between stress conditions and the desired increase in bioproduct synthesis is a complex subject.

Zeaxanthin belongs to the group of xanthophyll carotenoids and predominately accumulates in the human eye in the area of the macula lutea of the retina [75,76]. Therefore, this potent antioxidative substance counteracts age-related eye disorders and constitutes a very prominent exogenous (dietary) component, which is also used for food supplementation and pharmaceutical applications. Importantly, some mutant of *D. tertiolecta* (*mp3*, previously known as *mut12*) is able to hyperaccumulate zeaxanthin at levels up to 15% (per cell at ~8.8% NaCl) higher than those in the parent zeaxanthin-accumulating mutant strain *zea1* [75]. Generally, wild type *Dunaliella* are much poorer producers of zeaxanthin (regardless of salinity) than *zea1* strains [9,75]. It should be highlighted that extremely high salinity (from 17.5% NaCl) decreases maximal zeaxanthin synthesis in *D. tertiolecta* cells of *mp3* and *zea1* mutants as well as cell density [75]. Accordingly, β-carotene accumulation is also sensitive to hypersaline conditions (the drop is very pronounced at 14.6%, 17.5%, and 20.4% NaCl) [20]. The induction of glycerol and lipid biosynthesis (including triacylglycerides) in *Dunaliella* cells is also regulated by the salinity gradient [26,37]. The lipid fraction of *D. tertiolecta* may increase with increasing salinity (from ~3% to 5.8%), but NaCl concentrations higher than 5.8% start to impede further

cell growth, which is drastically limited in the presence of ~11.7% NaCl [26]. Analogously, in *D. salina*, the maximal level of glycerol was found in the presence of ~11.7% NaCl, while 17.5–23.4% NaCl was clearly less optimal for microalgal growth [37]. The same is true for phytosterols produced by *D. tertiolecta* and *D. salina*, which were found to show significantly decreased levels with increasing salinity (NaCl concentration higher than 3.5% may limit sterol accumulation) [77]. Phytosterols are another group of functional ingredients with multiple beneficial effects on health, e.g., ergosterol, which is one of the most abundantly produced sterols in *D. tertiolecta* and *D. salina*, is provitamin D2. Moreover, phytosterols of different origins can minimize the risk of certain diseases, such as various types of cancer and some cases of heart failure [67,78].

In summary, depending on the direction and scale of bioproduction, the culture conditions of microalgae have to be precisely adjusted (Fig. 2). Improvements in the extraction methods applied to obtain biomolecules from *Dunaliella* cells are also expected.

6. Nucleotide metabolism in plants, including microalgae such as *Dunaliella* spp.

To achieve a detailed understanding of *Dunaliella* spp. growth, it is very important to analyze the general metabolism and distribution of various nucleotides in the cells of these algae. Purine nucleotide channeling is critical for cell survival [79]. In turn, accessibility to phosphorus affects intracellular nucleotide pools in eukaryotes, including phytoplankton, and thus influences their growth rate [80]. Mitochondrial OXPHOS delivers the majority of ATP for energy-consuming processes in heterotrophs; however, autotrophs can also rely on chloroplasts to obtain ATP. Energy transfer between mitochondria and plastids (photosynthetic and nonphotosynthetic) enables the optimization of various processes, such as carbon fixation and growth, in terrestrial plants and algae [81,82]. Importantly, ATP and GTP are not only equivalents of “power currency” in living systems, but among others, they also serve as building blocks for RNA and can donate a phosphate group to other nucleotides (less phosphorylated) [83,84]. In turn, monophosphate nucleotides may play a role in phosphorus storage under conditions of phosphorus deficiency in marine eukaryotic phytoplankton [80]. The translocation of adenine nucleotides across mitochondrial and nonmitochondrial membranes in algae/plants is mediated by different specialized carrier proteins [79,85,86]. The passage of guanine nucleotides through lipid bilayers is generally still incompletely understood and has been only partly established for some species of nonautotrophic and autotrophic unicellular eukaryotes [83,86–89]. Nucleic acid biosynthesis in complex plastids of microalgae may depend on transporters with broad substrate specificity for both ribo- and deoxyribonucleoside triphosphates [79,86,87,89]. Intense metabolic activity (involving mitochondria) that is necessary for the rapid cell division of plants, including microalgae, also recruits transphosphorylation enzymes [81,90–93]. The rates of OXPHOS and different transphosphorylation reactions may also be positively related in cyanobacteria [94]. In this context, the key enzyme is considered to be NDPK, located in the cytosol and organelles [85,90,95]. This bivalent metal cation-dependent phosphotransferase acts as an oligomeric complex (usually hexamer) with broad substrate specificity [96–98] and is ubiquitous in different forms of life, both prokaryotic and eukaryotic, from bacteria to humans [90,95,99]. NDPK performs terminal γ-phosphate group exchange between donor nucleoside triphosphate and acceptor nucleoside diphosphate *via* a “ping-pong” mechanism, in which transient phosphoenzyme (autophosphorylated intermediate) exist [e.g., ATP (enzyme~P) + GDP → ADP + GTP (free enzyme)] [96,97]. Both steps of enzymatic catalysis may require bivalent metal cations, such as Mg^{2+}. Consequently, NDPK is able to stimulate oxygen-dependent respiration in different unicellular and multicellular organisms through delivery of ADP to the OXPHOS apparatus [81,91,100–102]. It is accentuated by the observation that NDPKs and mitochondrial ADP/ATP carriers (importing ADP into the matrix and exporting ATP into the intermembrane space) in plants may be physiological interactants (directly associated) [103]. This possibility is further supported by the fact that changes in the expression level of plant NDPK correlate, for example, with oxygen uptake and tricarboxylic acid cycle activity and, thus, respiration, carbon metabolism and growth [91]. Namely, the overexpression of NDPK alone may positively affect these apparent metabolic rate parameters, promoting the recycling of ADP molecules and a high ATP turnover rate. In contrast, O_2 consumption by mitochondria isolated from unicellular eukaryotes without active NDPK (i.e., a yeast mutant) is decreased [101]. OXPHOS inhibition by carboxyatractyloside (inhibitor of mitochondrial ADP/ATP carrier) and/or oligomycin (inhibitor of F_OF_1 ATP synthase), which block very important targets for ADP (generated, for example, by NDPK), also constrain the capacity of the respiratory chain [100–102]. Plants generally possess four types of NDPK isoforms, among which the cytosol (predominantly) and the nucleus and peroxisomes (to a lesser extent) are the environments of type I NDPKs (products of *ndpk1* and some *ndpk4* genes); type II NDPKs (products of *ndpk2* genes) are deposited in the chloroplast stroma; type III NDPKs (products of *ndpk3* and some *ndpk4* genes) are located in both the mitochondrial intermembrane space and the thylakoid lumen; and type IV NDPKs (products of *ndpk5* genes) constitute a quite novel group and are currently not well understood (e.g., predicted to potentially occur in the endoplasmic reticulum) [85,90,95].

NDPK also plays a protective role against multiple stresses, such as cold stress, oxidative stress, salt stress, and herbicides, as this enzyme positively contributes to the downregulation of the cellular redox state [104–106]. In plants, this kinase may also help to survive transient contamination with heavy metal ions, such as Ni^{2+}, as it can show a strong preference for phosphorylating ADP [96]. NDPK-dependent compensation for ATP (e.g., in the roots of *Alyssum murale* M. Bieberstein 1808 — metal hyperaccumulating plant) may regulate the pool of free histidine (at the initial and ATP-dependent step of its biosynthetic pathway), which is crucial for nickel tolerance. Free histidine is considered a high-affinity nitrogen-containing ligand of Ni^{2+} in some representatives of the *Alyssum* genus [107]. Aluminum ion resistance is also strongly correlated with substantially increased activity of NDPK in the root tips of some wheat strains [108]. Curiously, it has been shown in plants (potato roots) that higher expression of cytosolic NDPK1 translates to an increase in ROS (seen as a side effect of an increased metabolic rate), but without harmful effects [91]. However, the overexpression of NDPK2 in *Arabidopsis thaliana* (Linnaeus) Heynhold 1842 may counteract elevated ROS levels [104]. Conversely, the elimination of NDPK2 (in *A. thaliana* knockout mutants) favors a burst of ROS [97,104]. The mechanism involving NDPK2 probably relies on direct interaction with oxidative stress-sensitive reporters (i.e., specific binding to certain mitogen-activated protein kinases within the phosphorylation cascade targeting stress-response genes) [104]. Moreover, wild-type plants show substantially increased transcription of different NDPK isoforms after exogenous ROS (H_2O_2) exposure, and in the case of NDPK2, the response is the strongest at the earliest time point. NDPK2 (arabidopsis/tobacco) also plays a role in the context of salt sensitivity [97,106], as does NDPK1 (rice) [109]. Salinity can even increase the expression of NDPK (rice/tall fescue) [109,110] and the affinity of this kinase (barley) toward ATP substrates [98].

The mechanism revealed in plants is analogous to that in green algae, as mitochondrial NDPK of halotolerant *D. salina* is among the proteins increased by salinity shock [22]. Interestingly, in *Dunaliella* cells the expression of NDPK after salt stress is positively coupled with the upregulation of energy metabolism proteins (i.e., with some subunits of mitochondrial respiratory chain complex I and F_OF_1 ATP synthase). Generally, increases in the levels of proteins that are assembled into the OXPHOS apparatus may be attributed to early salinity-responsive pathways in *D. salina* [111]. The functional coexpression of mitochondrial NDPK and some elements of the OXPHOS system may indicate an essential interrelationship between energy conversion

(transphosphorylation) and membrane-associated energy transduction processes in *Dunaliella* cells. Furthermore, in stressed plants (including salt stress), upregulated expression of NDPK2 (arabidopsis) [104,105] and other NDPKs (rice/tall fescue) [109,110] may positively overlap with elevated expression of a variety of antioxidant genes, encoding products such as catalase, peroxidase, and thioredoxin. On the other hand, the increased production of plant NDPK2 (tobacco) may constitute a compensatory change when cytosolic ascorbate peroxidase is suppressed [106]. This kind of response may significantly increase cell viability under salt stress. Intriguingly, ascorbate peroxidase of *D. salina* belongs to salinity shock-decreased proteins [22]. To address this topic further, NDPK1-catalase functional heterocomplexes were identified in plants (arabidopsis) [112]. Therefore, all of these molecular and physiological relationships require more detailed studies to reveal common and complex strategies in all autophototrophic eukaryotes.

The importance of NDPKs to energy status may not be conserved in all eukaryotes. The lack of this enzyme (encoded by a single gene) only slightly affects the viability of the yeast *S. cerevisiae*, which can be grown successfully [84,101]. In contrast, the absence of NDPK3 may be lethal in plants (e.g., arabidopsis) [85]. This would be not surprising, as NDPK3 isoforms in plant chloroplasts, which generate GTP (e.g., for specific GTPases residing in the thylakoid lumen), may indirectly regulate the function and dynamics of the photosystem II complex. The currently known NDPK of *D. tertiolecta* is cognate to plant NDPK3 [90,113]. Thus far, this kinase has been identified only in *Dunaliella* mitochondria, revealing the first confirmed NDPK in this organelle in algae (Fig. 1) [22,113]. Strikingly, the long cleavable N-terminal mitochondrial targeting sequence of *Dunaliella* NDPK is recognized by yeast mitochondria [113]. The biochemical and structural properties of NDPK from *D. tertiolecta* (e.g., broad substrate specificity and hexameric organization) are quite concordant with homologs of other species [114]. The activity of NDPK in microalgae (marine diatoms) was analyzed in association with the cell growth rate over a light gradient [93]. There are some indications that the activity of this kinase is significantly and positively correlated with the growth rates of *Thalassiosira pseudonana* Hasle and Heimdal 1970. It must be noted that as the key auxiliary enzyme with constant activity in green algae (*Tetradesmus obliquus* (Turpin) M. J. Wynne 2016), NDPK is implicated in deoxyribonucleoside triphosphates biosynthesis [92]. In addition, NDPK supports motility, as the flagellar radial spokes of microalgae (*Chlamydomonas reinhardtii* P. A. Dangeard 1888) contain this kinase [115,116]. The possibility cannot be excluded that the axonemes of *Dunaliella* spp. also contain specific NDPKs, which could deliver nucleotides other than ATP (probably GTP) to regulate motor function. The investigation of nucleotide metabolism in microalgae is therefore an exciting future direction for increasing knowledge about processes such as energy provision in salinized environments.

7. Conclusions

The various potential uses of *Dunaliella* spp. in science, industry and medicine are certainly open questions (Fig. 3). A more detailed analysis of nucleotide metabolism, including mitochondrial NDPK, will aid in better understanding the specific nature of these microalgae, for example, regarding their reproduction and distribution patterns. Furthermore, special interest should be focused on abiotic stress factors that increase the production of bioactive compounds that are valuable for various consumers because changeable habitat/culture conditions, such as salinity fluctuations, apparently affect energy conversion/transduction processes in *D. salina* [22,111]. The modulation of mitochondria, in which proteins involved in nucleotide turnover are upregulated, may be critical for the survival of *Dunaliella* spp. in the environment. The optimization of growth conditions followed by the moderation of massive cultivation costs will contribute to the more common use of *Dunaliella*-based products to the benefit of mankind. Currently, permanent access to freshwater resources is becoming a major problem around the world, notably in regions with annual temperature increases and high sensitivity to desertification. In the near future, in many countries, plant cultivation and livestock breeding may prove impossible because of potable water deficits. The mass cultures of *Dunaliella* spp. in saline habitats that are unfavorable for many other organisms present great potential for the use of "wasteland" areas. Therefore, *Dunaliella* spp. aquafarming, especially in natural seawater, is noncompetitive with freshwater-dependent industry, land plant agriculture, and animal husbandry [75]. Finally, medical facilities may benefit from ingredients extracted from microalgae, such as various types of carotenes and their derivatives, for the treatment of neoplastic changes and pulmonary and cardiovascular diseases. Moreover, a lower price and widespread availability of carotenoids for inclusion in regular, balanced diets may solve some vision problems (including those in children) that occur mainly in developing countries [117]. Curiously, *D. salina* carotenoids can also lower virus titers in mammalian cells (probably by counteracting increases in viral replication) [118]. In the context of the coronavirus pandemic, the application of *Dunaliella* spp. bioproducts could support clinical therapy against this pathogen to overcome the risk of postinfection side effects, such as respiratory system dysfunction and reinfection.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this work.

CRediT authorship contribution statement

Andrzej M. Woyda-Ploszczyca: Project administration, Funding acquisition, Conceptualization, Supervision, Methodology, Visualization, Writing – original draft, Writing – review & editing, Validation. **Andrzej S. Rybak:** Methodology, Visualization, Writing – original draft, Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M. Guiry, G. Guiry, Algaebase, World-Wide Electron, Publ. Natl. Univ. Ireland, Galway, 2021. https://www.algaebase.org (accessed May 13, 2021).
[2] M.A. Buchheim, A.E. Kirkwood, J.A. Buchheim, B. Verghese, W.J. Henley, Hypersaline soil supports a diverse community of *Dunaliella* (Chlorophyceae), J. Phycol. 46 (2010) 1038–1047, https://doi.org/10.1111/j.1529-8817.2010.00886.x.
[3] I.T.K. Ru, Y.Y. Sung, M. Jusoh, M.E.A. Wahid, T. Nagappan, *Chlorella vulgaris*: a perspective on its potential for combining high biomass with high value bioproducts, Appl. Phycol. 1 (2020) 2–11, https://doi.org/10.1080/26388081.2020.1715256.
[4] G. Randrianarison, M.A. Ashraf, Microalgae: a potential plant for energy production, Geol. Ecol. Landscapes 1 (2017) 104–120, https://doi.org/10.1080/24749508.2017.1332853.
[5] R. Raja, S. Hemaiswarya, R. Rengasamy, Exploitation of *Dunaliella* for β-carotene production, Appl. Microbiol. Biotechnol. 74 (2007) 517–523, https://doi.org/10.1007/s00253-006-0777-8.
[6] A.C. Guedes, F.X. Malcata, Nutritional value and uses of microalgae in aquaculture, in: Aquaculture, InTech, 2012, pp. 59–78.
[7] Algae products market size, analysis, trends, growth and forecast to 2027. https://www.credenceresearch.com/report/algae-products-market, 2021 (accessed May 13, 2021).

[8] A. Hosseini Tafreshi, M. Shariati, *Dunaliella* biotechnology: methods and applications, J. Appl. Microbiol. 107 (2009) 14–35, https://doi.org/10.1111/j.1365-2672.2009.04153.x.

[9] A. Ben-Amotz, J.E.W. Polle, D.V.S. Rao, The Alga *Dunaliella*: Biodiversity, Physiology, Genomics and Biotechnology, 1st ed., CRC Press, 2009.

[10] R.W. Butcher, An Introductory Account of the Smaller Algae of British Coastal Waters: Part I: Introduction and Chlorophyceae, 1st ed., Fisheries Investigations, London, 1959.

[11] M.A. Borowitzka, C.J. Siva, The taxonomy of the genus *Dunaliella* (*Chlorophyta*, *Dunaliellales*) with emphasis on the marine and halophilic species, J. Appl. Phycol. 19 (2007) 567–590, https://doi.org/10.1007/s10811-007-9171-x.

[12] N. Massyuk, Morphology, Taxonomy, Ecology and Geographic Distribution of the Genus *Dunaliella* Teod. and Prospects for Its Potential Utilization, Naukova Dumka, Kiev, 1973.

[13] N. Bitterman, Y. Melamed, A. Ben-Amotz, β-Carotene and CNS oxygen toxicity in rats, J. Appl. Physiol. 76 (1994) 1073–1076, https://doi.org/10.1152/jappl.1994.76.3.1073.

[14] J.E.W. Polle, R. Roth, A. Ben-Amotz, U. Goodenough, Ultrastructure of the green alga *Dunaliella salina* strain CCAP19/18 (*Chlorophyta*) as investigated by quick-freeze deep-etch electron microscopy, Algal Res. 49 (2020), 101953, https://doi.org/10.1016/j.algal.2020.101953.

[15] J.E.W. Polle, E.S. Jin, A. Ben-Amotz, The alga *Dunaliella* revisited: looking back and moving forward with model and production organisms, Algal Res. 49 (2020), 101948, https://doi.org/10.1016/j.algal.2020.101948.

[16] A. Oren, The ecology of *Dunaliella* in high-salt environments, J. Biol. Res. 21 (2014) 23, https://doi.org/10.1186/s40709-014-0023-y.

[17] A. Oren, A hundred years of *Dunaliella* research: 1905–2005, Sal. Syst. 1 (2005) 2, https://doi.org/10.1186/1746-1448-1-2.

[18] Y. Sui, S.E. Vlaeminck, *Dunaliella* microalgae for nutritional protein: an undervalued asset, Trends Biotechnol. 38 (2020) 10–12, https://doi.org/10.1016/j.tibtech.2019.07.011.

[19] A.J. Liska, A. Shevchenko, U. Pick, A. Katz, Enhanced photosynthesis and redox energy production contribute to salinity tolerance in *Dunaliella* as revealed by homology-based proteomics, Plant Physiol. 136 (2004) 2806–2817, https://doi.org/10.1104/pp.104.039438.

[20] M.H. Liang, Y.F. Hao, Y.M. Li, Y.J. Liang, J.G. Jiang, Inhibiting lycopene lyclases to accumulate lycopene in high β-carotene-accumulating *Dunaliella bardawil*, Food Bioprocess Technol. 9 (2016) 1002–1009, https://doi.org/10.1007/s11947-016-1681-6.

[21] K.W.M. Tan, H. Lin, H. Shen, Y.K. Lee, Nitrogen-induced metabolic changes and molecular determinants of carbon allocation in *Dunaliella tertiolecta*, Sci. Rep. 6 (2016) 1–13, https://doi.org/10.1038/srep37235.

[22] S. Wei, Y. Bian, Q. Zhao, S. Chen, J. Mao, C. Song, K. Cheng, Z. Xiao, C. Zhang, W. Ma, H. Zou, M. Ye, S. Dai, Salinity-induced palmella formation mechanism in halotolerant algae *Dunaliella salina* revealed by quantitative proteomics and phosphoproteomics, Front. Plant Sci. 8 (2017) 810, https://doi.org/10.3389/fpls.2017.00810.

[23] M. Ginzburg, *Dunaliella*: a green alga adapted to salt, Adv. Bot. Res. 14 (1988) 93–183, https://doi.org/10.1016/S0065-2296(08)60271-2.

[24] H. Gimmler, Acidophilic and acidotolerant algae, in: L. Rai, J. Gaur (Eds.), Algal Adapt. to Environ. Stress, Springer, Berlin, Heidelberg, 2001, pp. 259–290, https://doi.org/10.1007/978-3-642-59491-5_9.

[25] P. Assunção, R. Jaén-Molina, J. Caujapé-Castells, A. de la Jara, L. Carmona, K. Freijanes, H. Mendoza, Phylogenetic position of *Dunaliella acidophila* (*Chlorophyceae*) based on ITS and rbcL sequences, J. Appl. Phycol. (2012), https://doi.org/10.1007/s10811-011-9676-1.

[26] M. Takagi, T. Yoshida Karseno, Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells, J. Biosci. Bioeng. 101 (2006) 223–226, https://doi.org/10.1263/jbb.101.223.

[27] H. Tang, N. Abunasser, M.E.D. Garcia, M. Chen, K.Y. Simon Ng, S.O. Salley, Potential of microalgae oil from *Dunaliella tertiolecta* as a feedstock for biodiesel, Appl. Energy 88 (2011) 3324–3330, https://doi.org/10.1016/j.apenergy.2010.09.013.

[28] A.M. Lakaniemi, V.M. Intihar, O.H. Tuovinen, J.A. Puhakka, Growth of *Dunaliella tertiolecta* and associated bacteria in photobioreactors, J. Ind. Microbiol. Biotechnol. 39 (2012) 1357–1365, https://doi.org/10.1007/s10295-012-1133-x.

[29] P.I. Gómez, M.A. González, The effect of temparature and irradiance on the growth and carotenogenic capacity of seven strains of *Dunaliella salina* (*Chlorophyta*) cultivated under laboratory conditions, Biol. Res. 38 (2005) 151–162, https://doi.org/10.4067/s0716-97602005000200005.

[30] A.J. Aasen, K.E. Eimhjellen, S. Liaaen-Jensen, An extreme source of β-carotene, Acta Chem. Scand. 23 (1969) 2544–2545, https://doi.org/10.3891/acta.chem.scand.23-2544.

[31] A. Ben-Amotz, A. Katz, M. Avron, Accumulation of β-carotene in halotolerant algae: purification and characterization of β-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae), J. Phycol. 18 (1982) 529–537, https://doi.org/10.1111/j.1529-8817.1982.tb03219.x.

[32] G. Kumar, A. Shekh, S. Jakhu, Y. Sharma, R. Kapoor, T.R. Sharma, Bioengineering of microalgae: recent advances, perspectives, and regulatory challenges for industrial application, Front. Bioeng. Biotechnol. 8 (2020) 914, https://doi.org/10.3389/fbioe.2020.00914.

[33] G. Tang, P.M. Suter, Vitamin A, nutrition, and health values of algae: *Spirulina*, *Chlorella*, and *Dunaliella*, J. Pharm. Nutr. Sci. 1 (2011) 111–118, https://doi.org/10.6000/1927-5951.2011.01.02.04.

[34] J. García, M. de Vicente, B. Galán, Microalgae, old sustainable food and fashion nutraceuticals, Microb. Biotechnol. 10 (2017) 1017–1024, https://doi.org/10.1111/1751-7915.12800.

[35] M. Mourelle, C. Gómez, J. Legido, The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy, Cosmetics 4 (2017) 46, https://doi.org/10.3390/cosmetics4040046.

[36] F. El-Baz, S. Abdo, A. Hussein, Microalgae *Dunaliella salina* for use as food supplement to improve pasta quality, Int. J. Pharm. Sci. Rev. Res. 46 (2017) 45–51.

[37] A. Ben-Amotz, I. Sussman, M. Avron, Glycerol production by *Dunaliella*, in: New Trends Res. Util. Sol. Energy Biol. Syst., Birkhäuser Basel, 1982, pp. 55–58, https://doi.org/10.1007/978-3-0348-6305-6_12.

[38] H.H. Chen, L.L. Xue, M.H. Liang, J.G. Jiang, Sodium azide intervention, salinity stress and two-step cultivation of *Dunaliella tertiolecta* for lipid accumulation, Enzym. Microb. Technol. 127 (2019) 1–5, https://doi.org/10.1016/j.enzmictec.2019.04.008.

[39] L.L. Xue, J.G. Jiang, Cultivation of *Dunaliella tertiolecta* intervened by triethylamine enhances the lipid content, Algal Res. 25 (2017) 136–141, https://doi.org/10.1016/j.algal.2017.04.019.

[40] Z.J. Qian, K.H. Kang, B.M. Ryu, Microalgae-derived toxic compounds, in: Handb. Mar. Microalgae Biotechnol. Adv., Elsevier Inc., 2015, pp. 527–537.

[41] S. Mokady, A. Abramovici, U. Cogan, The safety evaluation of *Dunaliella bardawil* as a potential food supplement, Food Chem. Toxicol. 27 (1989) 221–226, https://doi.org/10.1016/0278-6915(89)90159-2.

[42] X. Yanan, P.J. Harvey, Red light control of β-carotene isomerisation to 9-*cis* β-carotene and carotenoid accumulation in *Dunaliella salina*, Antioxidants 8 (2019) 148, https://doi.org/10.3390/antiox8050148.

[43] L. Jaime, J.A. Mendiola, E. Ibáñez, P.J. Martin-Álvarez, A. Cifuentes, G. Reglero, F.J. Señoráns, β-Carotene isomer composition of sub- and supercritical carbon dioxide extracts. Antioxidant activity measurement, J. Agric. Food Chem. 55 (2007) 10585–10590, https://doi.org/10.1021/jf0711789.

[44] G. Levin, M. Yeshurun, S. Mokady, *In vivo* antiperoxidative effect of 9-*cis* β-carotene compared with that of the all-*trans* isomer, Nutr. Cancer 27 (1997) 293–297, https://doi.org/10.1080/01635589709514540.

[45] G. Levin, S. Mokady, Antioxidant activity of 9-*cis* compared to all-*trans* β-carotene *in vitro*, Free Radic. Biol. Med. 17 (1994) 77–82, https://doi.org/10.1016/0891-5849(94)90009-4.

[46] J.T. Lin, Y.C. Lee, C.C. Hu, Y.C. Shen, F.J. Lu, D.J. Yang, Evaluation of carotenoid extract from *Dunaliella salina* against cadmium-induced cytotoxicity and transforming growth factor β1 induced expression of smooth muscle α-actin with rat liver cell lines, J. Food Drug Anal. 18 (2010) 301–306, https://doi.org/10.38212/2224-6614.2279.

[47] W.C. Chuang, Y.C. Ho, J.W. Liao, F.J. Lu, *Dunaliella salina* exhibits an antileukemic immunity in a mouse model of WEHI-3 leukemia cells, J. Agric. Food Chem. 62 (2014) 11479–11487, https://doi.org/10.1021/jf503564b.

[48] T. Weinrich, Y. Xu, C. Wosu, P.J. Harvey, G. Jeffery, Mitochondrial function, mobility and lifespan are improved in *Drosophila melanogaster* by extracts of 9-*cis*-β-carotene from *Dunaliella salina*, Mar. Drugs 17 (2019) 279, https://doi.org/10.3390/md17050279.

[49] K. Supamattaya, S. Kiriratnikom, M. Boonyaratpalin, L. Borowitzka, Effect of a *Dunaliella* extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*), in: Aquaculture, Elsevier, 2005, pp. 207–216, https://doi.org/10.1016/j.aquaculture.2005.04.014.

[50] R. Parker, Absorption, metabolism, and transport of carotenoids, FASEB J. Ser. Rev. 10 (1996) 542–551, https://doi.org/10.1096/fasebj.10.5.8621054.

[51] F.J. Ruperez, D. Garcia-Martinez, B. Baena, N. Maeso, M. Vallejo, S. Angulo, A. Garcia, E. Ibañez, F.J. Señorans, A. Cifuentes, C. Barbas, *Dunaliella salina* extract effect on diabetic rats: metabolic fingerprinting and target metabolite analysis, J. Pharm. Biomed. Anal. 49 (2009) 786–792, https://doi.org/10.1016/j.jpba.2008.12.041.

[52] M.T. Khayyal, F.K. El-Baz, M.R. Meselhy, G.H. Ali, R.M. El-Hazek, Intestinal injury can be effectively prevented by *Dunaliella salina* in gamma irradiated rats, Heliyon 5 (2019), https://doi.org/10.1016/j.heliyon.2019.e01814.

[53] F.K. El-Baz, H.F. Aly, H.I. Abd-Alla, The ameliorating effect of carotenoid rich fraction extracted from *Dunaliella salina* microalga against inflammation-associated cardiac dysfunction in obese rats, Toxicol. Rep. 7 (2020) 118–124, https://doi.org/10.1016/j.toxrep.2019.12.008.

[54] F.K. El-Baz, S.I. Ali, M. Basha, A.A. Kassem, R.N. Shamma, R. Elgohary, A. Salama, Design and evaluation of bioenhanced oral tablets of *Dunaliella salina* microalgae for treatment of liver fibrosis, J. Drug Deliv. Sci. Technol. (2020), 101845, https://doi.org/10.1016/j.jddst.2020.101845.

[55] H. Nagasawa, Y. Fujii, Y. Kageyama, T. Segawa, A. Ben-Amotz, Suppression by beta-carotene-rich algae *Dunaliella bardawil* of the progression, but not the development, of spontaneous mammary tumours in SHN virgin mice, Anticancer Res. 11 (1991) 713–717.

[56] Y. Fujii, S. Sakamoto, A. Ben-Amotz, H. Nagasawa, Effects of beta-barotene-rich algae *Dunaliella bardawil* on the dynamic changes of normal and neoplastic mammary cells and general metabolism in mice, Anticancer Res. 13 (1993) 389–393.

[57] F. Elleuch, P. Baril, M. Barkallah, F. Perche, S. Abdelkafi, I. Fendri, C. Pichon, Deciphering the biological activities of *Dunaliella* sp. aqueous extract from stressed conditions on breast cancer: from *in vitro* to *in vivo* investigations, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21051719.

[58] F.K. El-Baz, H.F. Aly, A.A.A. Salama, Toxicity assessment of the green *Dunaliella salina* microalgae, Toxicol. Rep. 6 (2019) 850–861, https://doi.org/10.1016/j.toxrep.2019.08.003.

[59] V. Pasquet, P. Morisset, S. Ihammouine, A. Chepied, L. Aumailley, J.B. Berard, B. Serive, R. Kaas, I. Lanneluc, V. Thiery, M. Lafferriere, J.M. Piot, T. Patrice, J. P. Cadoret, L. Picot, Antiproliferative activity of violaxanthin isolated from bioguided fractionation of *Dunaliella tertiolecta* extracts, Mar. Drugs 9 (2011) 819–831, https://doi.org/10.3390/md9050819.

[60] E. Mo, E. Ma, Anticancer effect of *Dunaliella salina* under stress and normal conditions against skin carcinoma cell line A431 *in vitro*, Iran. J. Fish. Sci. 11 (2012) 283–293.

[61] M.J. Sheu, G.J. Huang, C.H. Wu, J.S. Chen, H.Y. Chang, S.J. Chang, J.G. Chung, Ethanol extract of *Dunaliella salina* induces cell cycle arrest and apoptosis in A549 human non-small cell lung cancer cells, In Vivo (Brooklyn) 22 (2008) 369–378.

[62] A.K. Singh, R. Tiwari, V.K. Singh, P. Singh, S.R. Khadim, U. Singh, V. Srivastava Laxmi, S.H. Hasan, R.K. Asthana, Green synthesis of gold nanoparticles from *Dunaliella salina*, its characterization and in vitro anticancer activity on breast cancer cell line, J. Drug Deliv. Sci. Technol. 51 (2019) 164–176, https://doi.org/10.1016/j.jddst.2019.02.023.

[63] A.K. Singh, R. Tiwari, V. Kumar, P. Singh, S.K. Riyazat Khadim, A. Tiwari, V. Srivastava, S.H. Hasan, R.K. Asthana, Photo-induced biosynthesis of silver nanoparticles from aqueous extract of *Dunaliella salina* and their anticancer potential, J. Photochem. Photobiol. B Biol. 166 (2017) 202–211, https://doi.org/10.1016/j.jphotobiol.2016.11.020.

[64] K. Korzeniowska, B. Górka, J. Lipok, P.P. Wieczorek, Algae and their extracts in medical treatment, in: Algae Biomass Charact. Appl., Springer International Publishing, 2018, pp. 73–87, https://doi.org/10.1007/978-3-319-74703-3_7.

[65] I. Neuman, H. Nahum, A. Ben-Amotz, Prevention of exercise-induced asthma by a natural isomer mixture of β-carotene, Ann. Allergy, Asthma Immunol. 82 (1999) 549–553, https://doi.org/10.1016/S1081-1206(10)63165-1.

[66] M.I. Khan, J.H. Shin, J.D. Kim, The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products, Microb. Cell Fact. 17 (2018) 36, https://doi.org/10.1186/s12934-018-0879-x.

[67] M. Plaza, M. Herrero, A. Alejandro Cifuentes, E. Ibáñez, Innovative natural functional ingredients from microalgae, J. Agric. Food Chem. 57 (2009) 7159–7170, https://doi.org/10.1021/jf901070g.

[68] K. Tsukahara, S. Sawayama, Liquid fuel production using microalgae, J. Japan Pet. Inst. 48 (2005) 251–259, https://doi.org/10.1627/jpi.48.251.

[69] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306, https://doi.org/10.1016/j.biotechadv.2007.02.001.

[70] O.K. Lee, A.L. Kim, D.H. Seong, C.G. Lee, Y.T. Jung, J.W. Lee, E.Y. Lee, Chemo-enzymatic saccharification and bioethanol fermentation of lipid-extracted residual biomass of the microalga, *Dunaliella tertiolecta*, Bioresour. Technol. 132 (2013) 197–201, https://doi.org/10.1016/j.biortech.2013.01.007.

[71] A.L. Chagas, A.O. Rios, A. Jarenkow, N.R. Marcílio, M.A.Z. Ayub, R. Rech, Production of carotenoids and lipids by *Dunaliella tertiolecta* using CO_2 from beer fermentation, Process Biochem. 50 (2015) 981–988, https://doi.org/10.1016/j.procbio.2015.03.012.

[72] A.A. Ramos, J. Polle, D. Tran, J.C. Cushman, E.-S. Jin, J.C. Varela, The unicellular green alga *Dunaliella salina* Teod. as a model for abiotic stress tolerance: genetic advances and future perspectives, Algae 26 (2011) 3–20, https://doi.org/10.4490/algae.2011.26.1.003.

[73] M. Herrero, L. Jaime, P.J. Martín-Álvarez, A. Cifuentes, E. Ibáñez, Optimization of the extraction of antioxidants from *Dunaliella salina* microalga by pressurized liquids, J. Agric. Food Chem. 54 (2006) 5597–5603, https://doi.org/10.1021/jf060546q.

[74] M. Herrero, E. Ibáñez, A. Cifuentes, G. Reglero, S. Santoyo, *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials, J. Food Prot. 69 (2006) 2471–2477, https://doi.org/10.4315/0362-028X-69.10.2471.

[75] M. Kim, J. Ahn, H. Jeon, E.S. Jin, A. Cutignano, G. Romano, Development of a *Dunaliella tertiolecta* strain with increased zeaxanthin content using random mutagenesis, Mar. Drugs 15 (2017), https://doi.org/10.3390/md15060189.

[76] Y. Zhang, Z. Liu, J. Sun, C. Xue, X. Mao, Biotechnological production of zeaxanthin by microorganisms, Trends Food Sci. Technol. 71 (2018) 225–234, https://doi.org/10.1016/j.tifs.2017.11.006.

[77] M. Francavilla, P. Trotta, R. Luque, Phytosterols from *Dunaliella tertiolecta* and *Dunaliella salina*: a potentially novel industrial application, Bioresour. Technol. 101 (2010) 4144–4150, https://doi.org/10.1016/j.biortech.2009.12.139.

[78] L. Novotny, F. Mahmoud, M. Abdel-Hamid, L. Hunakova, Anticancer potential of β-sitosterol, Int. J. Clin. Pharmacol. Pharmacother. 2 (2017), https://doi.org/10.15344/2456-3501/2017/129.

[79] I. Haferkamp, A.R. Fernie, H.E. Neuhaus, Adenine nucleotide transport in plants: much more than a mitochondrial issue, Trends Plant Sci. 16 (2011) 507–515, https://doi.org/10.1016/j.tplants.2011.04.001.

[80] E.B. Kujawinski, K. Longnecker, H. Alexander, S.T. Dyhrman, C.L. Fiore, S. T. Haley, W.M. Johnson, Phosphorus availability regulates intracellular nucleotides in marine eukaryotic phytoplankton, Limnol. Oceanogr. Lett. 2 (2017) 119–129, https://doi.org/10.1002/lol2.10043.

[81] S. Dorion, J. Rivoal, Plant nucleoside diphosphate kinase 1: a housekeeping enzyme with moonlighting activity, Plant Signal. Behav. 13 (2018), e1475804, https://doi.org/10.1080/15592324.2018.1475804.

[82] B. Bailleul, N. Berne, O. Murik, D. Petroutsos, J. Prihoda, A. Tanaka, V. Villanova, R. Bligny, S. Flori, D. Falconet, A. Krieger-Liszkay, S. Santabarbara, F. Rappaport, P. Joliot, L. Tirichine, P.G. Falkowski, P. Cardol, C. Bowler, G. Finazzi, Energetic coupling between plastids and mitochondria drives CO_2 assimilation in diatoms, Nature 524 (2015) 366–369, https://doi.org/10.1038/nature14599.

[83] A. Vozza, E. Blanco, L. Palmieri, F. Palmieri, Identification of the mitochondrial GTP/GDP transporter in *Saccharomyces cerevisiae*, J. Biol. Chem. 279 (2004) 20850–20857, https://doi.org/10.1074/jbc.M313610200.

[84] B. Amutha, D. Pain, Nucleoside diphosphate kinase of *Saccharomyces cerevisiae*, Ynk1p: localization to the mitochondrial intermembrane space, Biochem. J. 370 (2003) 805–815, https://doi.org/10.1042/BJ20021415.

[85] C. Spetea, B. Lundin, Evidence for nucleotide-dependent processes in the thylakoid lumen of plant chloroplasts — an update, FEBS Lett. 586 (2012) 2946–2954, https://doi.org/10.1016/j.febslet.2012.07.005.

[86] A. Gruber, I. Haferkamp, Nucleotide transport and metabolism in diatoms, Biomolecules 9 (2019) 761, https://doi.org/10.3390/biom9120761.

[87] M. Ast, A. Gruber, S. Schmitz-Esser, H.E. Neuhaus, P.G. Kroth, M. Horn, I. Haferkamp, Diatom plastids depend on nucleotide import from the cytosol, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 3621–3626, https://doi.org/10.1073/pnas.0808862106.

[88] E. Heinz, C. Hacker, P. Dean, J. Mifsud, A.V. Goldberg, T.A. Williams, S. Nakjang, A. Gregory, R.P. Hirt, J.M. Lucocq, E.R.S. Kunji, T.M. Embley, Plasma membrane-located purine nucleotide transport proteins are key components for host exploitation by microsporidian intracellular parasites, PLoS Pathog. 10 (2014), e1004547, https://doi.org/10.1371/journal.ppat.1004547.

[89] L. Chu, A. Gruber, M. Ast, S. Schmitz-Esser, J. Altensell, H.E. Neuhaus, P.G. Kroth, I. Haferkamp, Shuttling of (deoxy-) purine nucleotides between compartments of the diatom *Phaeodactylum tricornutum*, New Phytol. 213 (2017) 193–205, https://doi.org/10.1111/nph.14126.

[90] J. Hammargren, J. Sundström, M. Johansson, P. Bergman, C. Knorpp, On the phylogeny, expression and targeting of plant nucleoside diphosphate kinases, Physiol. Plant. 129 (2007) 79–89, https://doi.org/10.1111/j.1399-3054.2006.00794.x.

[91] S. Dorion, A. Clendenning, J. Rivoal, Engineering the expression level of cytosolic nucleoside diphosphate kinase in transgenic *Solanum tuberosum* roots alters growth, respiration and carbon metabolism, Plant J. 89 (2017) 914–926, https://doi.org/10.1111/tpj.13431.

[92] B. Klein, H. Follmann, Deoxyribonucleotide biosynthesis in green algae. S phase-specific thymidylate kinase and unspecific nucleoside diphosphate kinase in *Scenedesmus obliquus*, Zeitschr. Naturforsch. C 43 (1988) 377–385.

[93] J.A. Berges, P.J. Harrison, Relationship between nucleoside diphosphate kinase activity and light-limited growth rate in the marine diatom *Thalassiosira pseudonana* (*Bacillariophyceae*), J. Phycol. 29 (1993) 45–53, https://doi.org/10.1111/j.1529-8817.1993.tb00278.x.

[94] W.H. Nitschmann, G.A. Peschek, Oxidative phosphorylation and energy buffering in cyanobacteria, J. Bacteriol. 168 (1986) 1205–1211, https://doi.org/10.1128/jb.168.3.1205-1211.1986.

[95] S. Dorion, J. Rivoal, Clues to the functions of plant NDPK isoforms, Naunyn. Schmiedebergs. Arch. Pharmacol. 388 (2015) 119–132, https://doi.org/10.1007/s00210-014-1009-x.

[96] T. Yupsanis, L. Symeonidis, C. Vergidou, A. Siskos, S. Michailidou, A. Yupsani, Purification, properties and specificity of a NDP kinase from *Alyssum murale* grown under Ni^{2+} toxicity, J. Plant Physiol. 164 (2007) 1113–1123, https://doi.org/10.1016/j.jplph.2006.12.006.

[97] P.E. Verslues, G. Batelli, S. Grillo, F. Agius, Y.-S. Kim, J. Zhu, M. Agarwal, S. Katiyar-Agarwal, J.-K. Zhu, Interaction of SOS2 with nucleoside diphosphate kinase 2 and catalases reveals a point of connection between salt stress and H_2O_2 signaling in *Arabidopsis thaliana*, Mol. Cell. Biol. 27 (2007) 7771–7780, https://doi.org/10.1128/mcb.00429-07.

[98] M. Reuveni, Y. Epstein, D. Evnor, F.M. DuPont, Utilization of metabolic energy under NaCl stress: activity of nucleoside diphosphate kinase, J. Plant Physiol. 154 (1999) 789–794, https://doi.org/10.1016/S0176-1617(99)80259-4.

[99] D. Perina, M. Korolija, A. Mikoč, M. Halasz, M.H. Bosnar, H. Ćetković, Characterization of Nme5-like gene/protein from the red alga *Chondrus crispus*, Mar. Drugs 18 (2019) 13, https://doi.org/10.3390/md18010013.

[100] A.M. Woyda-Ploszczyca, W. Jarmuszkiewicz, Different effects of guanine nucleotides (GDP and GTP) on protein-mediated mitochondrial proton leak, PLoS One 9 (2014), e98969, https://doi.org/10.1371/journal.pone.0098969.

[101] A.M. Woyda-Ploszczyca, W. Jarmuszkiewicz, The significance of nucleoside diphosphate kinase (NDPK)-dependent transphosphorylation for ADP/ATP carrier (AAC)-mediated mitochondrial proton leak of yeast *Saccharomyces cerevisiae*, Purinergic Signal. 14 (2019), https://doi.org/10.1007/s11302-018-9637-0. S68–S68/S107–S107.

[102] A.M. Woyda-Ploszczyca, W. Jarmuszkiewicz, The conserved regulation of mitochondrial uncoupling proteins: from unicellular eukaryotes to mammals, Biochim. Biophys. Acta Bioenerg. 1858 (2017) 21–33, https://doi.org/10.1016/j.bbabio.2016.10.003.

[103] C. Knorpp, M. Johansson, A.M. Baird, Plant mitochondrial nucleoside diphosphate kinase is attached to the membrane through interaction with the adenine nucleotide translocator, FEBS Lett. 555 (2003) 363–366, https://doi.org/10.1016/S0014-5793(03)01288-2.

[104] H. Moon, B. Lee, G. Choi, D. Shin, D. Theertha Prasad, O. Lee, S.S. Kwak, D. Hoon Kim, J. Nam, J. Bahk, J. Chan Hong, S. Yeol Lee, M. Je Cho, C.Oh. Lim, D. J. Yun, NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 358–363, https://doi.org/10.1073/pnas.252641899.

[105] K.A. Yang, H. Moon, G. Kim, C.J. Lim, J.C. Hong, C.O. Lim, D.J. Yun, NDP kinase 2 regulates expression of antioxidant genes in *Arabidopsis*, Proc. Japan Acad. Ser. B Phys. Biol. Sci. 79 (2003) 86–91, https://doi.org/10.2183/pjab.79B.86.

[106] T. Ishikawa, Y. Morimoto, R. Madhusudhan, Y. Sawa, H. Shibata, Y. Yabuta, A. Nishizawa, S. Shigeoka, Acclimation to diverse environmental stresses caused by a suppression of cytosolic ascorbate peroxidase in tobacco BY-2 cells, Plant Cell Physiol. 46 (2005) 1264–1271, https://doi.org/10.1093/pcp/pci135.

[107] U. Krämer, J.D. Cotter-Howells, J.M. Charnock, A.J.M. Baker, J.A.C. Smith, Free histidine as a metal chelator in plants that accumulate nickel, Nature 379 (1996) 635–638, https://doi.org/10.1038/379635a0.

[108] J.J. Slaski, Effect of aluminium on calmodulin-dependent and calmodulin-independent NAD kinase activity in wheat (*Triticum aestivum* L.) root tips, J. Plant Physiol. 133 (1989) 696–701, https://doi.org/10.1016/S0176-1617(89)80075-6.

[109] S. Kawasaki, C. Borchert, M. Deyholos, H. Wang, S. Brazille, K. Kawai, D. Galbraith, H.J. Bohnert, Gene expression profiles during the initial phase of salt stress in rice, Plant Cell 13 (2001) 889–905, https://doi.org/10.1105/tpc.13.4.889.

[110] Z. Shahabzadeh, R. Darvishzadeh, R. Mohammadi, M. Jafari, Isolation, characterization, and expression profiling of nucleoside diphosphate kinase gene from tall fescue (*Festuca arundinaceous* Schreb.) (FaNDPK) under salt stress, Plant Mol. Biol. Rep. 38 (2020) 175–186, https://doi.org/10.1007/s11105-019-01183-0.

[111] Y. Wang, Y. Cong, Y. Wang, Z. Guo, J. Yue, Z. Xing, X. Gao, X. Chai, Identification of early salinity stress-responsive proteins in *Dunaliella salina* by isobaric tags for relative and absolute quantitation (iTRAQ)-based quantitative proteomic analysis, Int. J. Mol. Sci. 20 (2019) 599, https://doi.org/10.3390/ijms20030599.

[112] Y. Fukamatsu, N. Yabe, K. Hasunuma, *Arabidopsis* NDK1 is a component of ROS signaling by interacting with three catalases, Plant Cell Physiol. 44 (2003) 982–989, https://doi.org/10.1093/pcp/pcg140.

[113] M.I. Anderca, T. Furuichi, R. Pinontoan, S. Muto, Identification of a mitochondrial nucleoside diphosphate kinase from the green alga *Dunaliella tertiolecta*, Plant Cell Physiol. 43 (2002) 1276–1284, https://doi.org/10.1093/pcp/pcf155.

[114] M.I. Anderca, T. Furuichi, S. Muto, Mitochondrial NDP kinase from *Dunaliella tertiolecta* (*Chlorophyceae*, *Chlorophyta*), Phycol. Res. 51 (2003) 147–153, https://doi.org/10.1046/j.1440-1835.2003.00304.x.

[115] T. Watanabe, M. Flavin, Nucleotide metabolizing enzymes in *Chlamydomonas* flagella, J. Biol. Chem. 251 (1976) 182–192, https://doi.org/10.1016/s0021-9258(17)33943-1.

[116] R.S. Patel-King, O. Gorbatyuk, S. Takebe, S.M. King, Flagellar radial spokes contain a Ca^{2+}-stimulated nucleoside diphosphate kinase, Mol. Biol. Cell. 15 (2004) 3891–3902, https://doi.org/10.1091/mbc.E04-04-0352.

[117] S. Kamal, M. Junaid, A. Ejaz, I. Bibi, N. Bigiu, Eye sight and carotenoids, in: Carotenoids Struct. Funct. Hum. Body, Springer International Publishing, Cham, 2021, pp. 609–647, https://doi.org/10.1007/978-3-030-46459-2_19.

[118] H.W. Lin, Y.C. Chen, C.W. Liu, D.J. Yang, S.Y. Chen, T.J. Chang, Y.Y. Chang, Regulation of virus-induced inflammatory response by *Dunaliella salina* alga extract in macrophages, Food Chem. Toxicol. 71 (2014) 159–165, https://doi.org/10.1016/j.fct.2014.05.026.